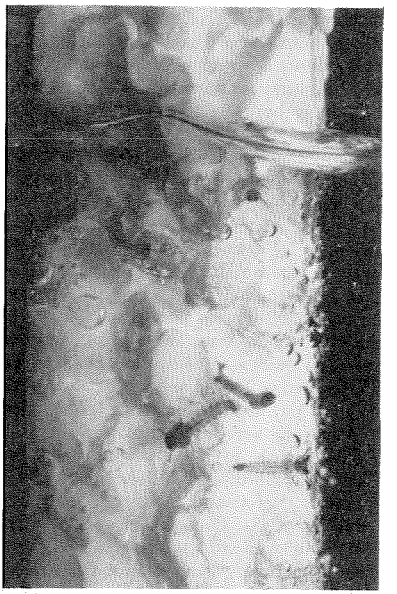

Figure 1. 4th instar larvae of *Coquillettidia perturbans* attached to styrofoam submerged in beaker.

which failed to pupate, all were still alive on June 11 (21 days), 2 survived until June 25 (35 days) and the last specimen died on June 29, after 39 days of attachment. At this point, observations were terminated.

Note: These observations were opportunistic and made with little deliberate care and under less than ideal experimental conditions. However, because of the relative success in attachment, survival and development of the specimens in Beaker B when compared to Beaker A, it seems possible that differences between shape, form and position of the styrofoam might have been significant.

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LEG FRACTURE IN ADULT MOSQUITOES INDUCED BY BIORESMETHRIN[1]

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The efficacy of mosquito adulticides is usually evaluated on the basis of their acute toxicity. However, toxicants may cause ancillary effects which, though non-lethal, may be detrimental to the target species. Recently this vividly came to our attention, when topical application of bioresmethrin (*trans*-resmethrin) to adult mosquitoes caused the complete fracture of one or more legs. Conceivably individuals with multiple leg fracture may be unsuccessful in flight, host seeking, and oviposition, especially those with one or no leg at all. The purpose of this communication is to describe this phenomenon and alert others to its possible significance.

Leg fracture occurs at the trochanterofemoral joint, which is similar to a ball and socket type (Christophers 1960). It has been observed in both sexes of the following species: *Aedes aegypti* (Linnaeus), *Ae. triseriatus* (Say), *Ae. sollicitans* (Walker), *Ae. canadensis* (Theobald), *Anopheles quadrimaculatus* Say, *Culex pipiens* Linnaeus, *Cx. salinarius* Coquillett and *Culiseta melanura* (Coquillett). Fracture occurs as early as 2 min posttreatment; it appears to be a consequence of strained leg flexion, because treated mosquitoes without leg-substrate contact also lose their legs.

In these current studies topical application

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was conducted with a microapplicator (Instrumentation Specialties Co., Nebraska) by means of a 0.25 ml syringe fitted with a 27 gauge hypodermic needle bent at right angles and its tip blunted. Prior to treatment, 10 female mosquitoes were aspirated into a 9 cm plastic petri dish through a hole made in the cover. The base of the petri dish was lined with a piece of filter paper. A 2 cm segment of cotton roll was partially inserted into the hole on the cover and moistened with 10% sucrose solution.

Carbon dioxide anesthesia was employed in order to facilitate handling during treatment. Prior to treatment, each petri dish was placed in the anesthesia chamber and a slow flow of CO_2 was piped into the chamber. The cover of the dish was slightly raised so that CO_2 permeated the petri dish as it displaced air. After 5–10 sec, the immobilized mosquitoes were ready for treatment. Each individual was picked up by the legs with a pair of flexible micro-dissection forceps (Turtox, New York) and positioned to receive 0.5 μl of the insecticide-acetone solution on the pleural area of the thorax.

In Table 1 are summarized the results with *Ae. aegypti* treated topically with bioresmethrin. The LD_{50} is 1.07 ng/individual (fiducial limits 0.97–1.18; Y = 5. 828X − 0.988). The incidence of leg fracture apparently is inversely related to the lethality level of treatment. At levels causing complete mortality (2.5 ng and higher) fracture was less than at partially lethal levels (0.7–1.5 ng) and at sublethal levels 0.25–0.50). A treatment of 0.1 ng was insufficient to cause significant fracture. These results may be explained by differences in symptomology of bioresmethrin poisoning at the various treatment levels. At the higher levels, treated mosquitoes remained on their dorsa, with their legs quivering. At the lower levels, the majority righted themselves and subsequently entered a stage of hyperactivity, when most fracture occurred. According to Camougis (1973), the action of pyrethrins on the arthropod nervous system proceeds in 2 steps, an initial excitation phase and a subsequent blocking phase, the duration of both phases varying inversely with concentration. At high treatment levels of bioresmethrin, rapid paralysis (blocking) apparently reduces the incidence of leg fracture.

Since the blocking action of allethrin is temperature dependent (Narahashi 1976), we also have examined the effect of temperature on mortality and leg fracture. Presented in Table 2 are results of an experiment wherein mosquitoes were immediately placed in posttreatment temperatures of 20°C or 28°C. After 24 hours at 20°C, those treated with 7.0 or 0.7 ng remained alive on their dorsa, with legs quivering; all those treated with 0.25 ng had righted themselves. In contrast, after 24 hours at 28°C, all treated with 7.0 ng were dead; the majority of those treated with 0.7 ng or 0.25 ng recovered within 24 hours. By 48 hours, the mortalities at respective treatment levels were greater at 20°C, but there were only slight differences in the incidence of leg fracture.

Table 1. Mortality and incidence of leg fracture of *Ae. aegypti* topically treated with bioresmethrin.[a, b]

Bioresmethrin, ng/individual	% mortality 48 h posttreatment	No. of legs lost/ 10 individuals, 48 h posttreatment
7.00	100	4.8
4.00	100	4.0
2.50	100	9.3
1.50	83.3	9.7
1.00	44.0	12.7
0.70	15.0	15.3
0.50	2.0	12.3
0.35	6.0	7.0
0.25	1.1	12.7
0.10	0.0	2.0
0.00	0.0	0.0

[a] Treated mosquitoes were maintained at 28°C.
[b] At least 30 females were tested at each dosage level.

Table 2. Effect of temperature on mortality and leg fracture of *Ae aegypti* topically treated with bioresmethrin.[a]

Temp.	Bioresmethrin ng/individual	% mortality 24 h	% mortality 48 h	No. of legs lost per 10 individuals[b] at 48 h
20°C	7.00	0	100	2.8
	0.70	0	83	10.2
	0.25	0	10	11.0
	0.0	0	0	0.0
28°C	7.00	100	100	4.8
	0.70	11.7	11.7	15.8
	0.25	0	0	12.3
	0.0	0	0	0.0

[a] Sixty females were treated for each dosage level.
[b] Based on all individuals examined.

The majority of treated mosquitoes lost 1–3 legs with a few losing all legs. When the survivors were exposed to a restrained guinea pig in a 2 ft^3 cage, those with no legs or only one leg were unable to fly and did not feed. Others with fewer missing legs did exhibit impaired flight patterns but succeeded in reaching and feeding on the host. Surprisingly, those mosquitoes with only 2 unilateral legs remaining were also successful in obtaining a blood meal. However, the laboratory conditions were relatively ideal, and under field conditions such mosquitoes would be less capable of sustained flight.

In previous field studies with resmethrin (Sutherland et al. 1980), we did not closely examine mosquitoes for leg fracture, but recently its occurrence has been confirmed. In the laboratory, leg fracture is not restricted to bioresmethrin but can also be induced by other pyrethroids such as fenvalerate, tetramethrin and American Cyanamid 222–705.

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